RNA Folding

2109-Pos Board B128
The Essential Adenosine Stacking in a Two-Base-Pair Minimal Kissing Complex
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A stable RNA helix requires at least three base pairs. Surprisingly, a tertiary kissing complex formed between two GACG hairpin loops contains only two GC pairs. In the NMR structure of this complex, the two flanking adenosines stack on the kissing GC pair. This observation raised a possibility that the 5'-dangling adenines contribute to the formation and stability of the kissing interaction. To test this hypothesis, we took a two-pronged approach to examine the effects of various mutational and chemical modifications of the flanking adenosines on the folding of the kissing complex. using mass spectrometry, we studied dimerization of various kissing hairpins. using optical tweezers, we monitored mechanical unfolding of intramolecular kissing complexes at single-molecule level. In both experiments, replacing either adenine with a uridine abolished the kissing interaction, suggesting that a minimal kissing complex must contain two GC pairs flanked by inter-strand stacking adenines. The stabilizing effect by the adenines can be explained by the fact that the stacking purine nucleobases shield the hydrogen bonds of the adjacent GC pairs, preventing them from fraying. Unlike in the context of secondary structure, the 5'-unpaired adenines in the tertiary structure are structurally constrained in a way that allows for effective stacking onto the adjacent base pairs.

2110-Pos Board B129
The Role of Ionic Strength and Ion Valence in RNA Collapse and Folding
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RNA folds efficiently in the presence of divalent ions, Mg2+ ions in particular. In fact, enhanced charge screening by divalent ions is a recurring theme in the study of nucleic acid electrostatics. Although site-specific ions have been identified in RNA crystal structures, the counterion atmosphere that screens the RNA charge is largely composed of a diffuse and mobile ion cloud. Here, we use Small Angle X-ray Scattering (SAXS), Fluorescence Correlation Spectroscopy (FCS) and microfluidic mixing kinetics to investigate the role of ionic strength and ion valence in the interactions, collapse and folding of small model RNA molecules. Our measurements suggest that in the presence of divalent ions the effective charge density near the RNA surface is smaller. We propose that a tighter localization of divalent ions leads to a larger degree of charge compensation and facilitates RNA folding.

2111-Pos Board B130
A Novel Bent Intermediate in the Dimerization of HIV-1 DIS RNA
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Dimerization Initiation Sequence (DIS) plays a crucial role in genome dimerization through formation of a ''kissing complex'' intermediate between two homologous DIS sequences. DIS is a conserved hairpin loop motif on the 5' UTR of the HIV-1 genome. This bimolecular kissing complex ultimately leads to the formation of an extended RNA duplex. Studying the mechanism and kinetics of this RNA interaction is the key to ex-ploiting DIS as a possible HIV drug target.
Here, we report a novel study that makes an important contribution to understanding the dimerization mechanism of HIV-1 RNA in vitro. This work employed single-molecule fluorescence resonance energy transfer (smFRET) to monitor the dimerization of minimal HIV-1 RNA sequence containing DIS. Most significantly, we observe a previously uncharacterized folding intermediate that plays a critical role in the dimerization mechanism. Our data reveal that dimerization involves three distinct steps in dynamic equilibrium and the equilibrium between the steps are regulated by Mg^{2+} ions. Two of the steps are identified as previously proposed structures: the kissing complex and the extended duplex. In addition to these, our data reveal a previously unobserved folding intermediate, consistent with a bent kissing complex conformation, similar to the TAR-TAT complex. Mutations of the highly conserved purines flanking the DIS loop destabilize this intermediate which indicates that these purines may play an important role in the HIV-1 RNA dimerization in vivo. The mechanistic insights gained from these experiments would represent significant progress in understanding the HIV-1 dimerization mechanism.

2112-Pos Board B131
Global Studies of Single-Stranded Nucleic Acid Conformation
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Unstructured regions of RNA molecules require flexibility to accomplish many biological tasks such as conformational switching and protein recognition. Due to its highly charged backbone, the flexibility of single-stranded RNA is influenced by counterions. In this presentation, we continue to explore RNA flexibility using single-stranded nucleic acid homopolymers as a model system [1]. We investigate the role of counterion valence in nucleic acid flexibility using a combination of small-angle X-ray scattering (SAXS) and single-molecule Förster resonance energy transfer (smFRET). We also study how charge-screening of these model systems are affected by mono- and divalent ions in competition. The results imply that various factors can alter the polymeric properties of unstructured nucleic acids, and may be important for tuning RNA conformational dynamics *in vivo*.
Reference: [1] Chen et al. PNAS 2012 109 (3) 799-804

2113-Pos Board B132
RNA Folding Landscapes in the Presence of Putrescine and Magnesium
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RNA folding in vivo is driven by a diverse set of interactions with cytoplasmic components. Of these interactions, the screening of negatively charged phosphates on the RNA backbone by cations is critical for folding to the native structure. Given their high intracellular concentrations, the positive charges of Mg^{2+} and putrescine (totaling 100mM and 60mM respectively) neutralize a large fraction of negative charges possessed by structured RNA. Using an experiment analogous to equilibrium dialysis we observe the competitive interactions of putrescine and Mg^{2+} in an RNA ion atmosphere for both native and intermediate states of RNAs. These interactions are related to a folding free energy landscape for three different RNAs. In the presence of Mg^{2+}, putrescine is found to be either stabilizing or destabilizing depending on the fold of the RNA. RNAs that chelate Mg^{2+} become destabilized in the presence of putrescine while non-chelators become more stabilized by putrescine. For some RNA molecules putrescine has an apparent synergistic effect on excess Mg^{2+} while other RNAs show an antagonistic effect. These results can be attributed to a difference in the manner in which putrescine and Mg^{2+} populate the ion atmosphere and affect RNA conformation.

2114-Pos Board B133
Structure and Functional Dynamics of Fluoride-Sensing Riboswitches
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Riboswitches are gene-regulatory RNA motifs located in the 5' untranslated regions of certain bacterial mRNA's. Riboswitches regulate gene expression by binding a metabolite related to the downstream gene, causing a conformational change that alters the accessibility of the gene for either transcription or translation. An important class of riboswitches that bind fluoride (F-) has been identified recently in bacteria and archaea, shedding light on how these organisms regulate internal fluoride concentrations to mitigate toxicity. The crystal structure of the fluoride riboswitch from Thermophilus petrophila shows a binding pocket in which the F- ion is coordinated by three Mg^{2+} ions. However, how ligand recognition and RNA folding are coupled to selectively encapsulate F- is not fully understood. Here, single-molecule TIRF microscopy and FRET are used to gain insights into the functional dynamics of fluoride riboswitches. Individual fluorescently-labelled fluoride riboswitches are immobilized on a quartz microscope slide, and the change in FRET efficiency between the fluorophors is used to study the ligand-binding mechanism and other cation- or denaturant-dependent structural transitions.

2115-Pos Board B134
Counterion-Dependent Folding of *Azoarcus* Ribozyme by SAXS Profile based Hybrid Simulation
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